Abstracts

1. FUNDAMENTAL STUDIES IN CHEMOTHERAPY OF TUBERCULOSIS

PART 64. STUDIES ON THE EFFECT OF SM-TREATMENT UPON TUBERCULOUS GUINEA PIGS PRETREATED WITH BCG (No. 1)

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A series of experiments was carried out in order to investigate the effect of streptomycin

Table 1. Schedule of experiment

Group			Number of guinea pigs	Days after BCG inoculation: 0	Days after $H_{37}Rv$ infection: 0	5	9 10	19	30
I		inoculated with BCG	4	↓	↓	×			
		not inoculated with BCG	2		↓	×			
II	treated	inoculated with BCG	4	↓	↓	< 5-day treatment >			×
		not inoculated with BCG	2		↓	< 5-day treatment >			×
	untreated	inoculated with BCG	4	↓	↓				×
		not inoculated with BCG	2		↓				×
		Control (without challenge) infection	4	↓					×
III	treated	inoculated with BCG	4	↓	↓		< 10-day treatment >		×
		not inoculated with BCG	2		↓		< 10-day treatment >		×
	untreated	inoculated with BCG	4	↓	↓				×
		not inoculated with BCG	2		↓				×
		Control (without challenge) infection	4	↓					×

1) ↓ inoculated with 0.1mg of BCG
↓ infected subcutaneously with 0.1mg of human tubercle bacilli $H_{37}Rv$
2) <·····> daily dose of SM : 40mg
3) × autopsied

(SM) upon guinea pigs infected with human tubercle bacilli "$H_{37}Rv$" after pretreatment with BCG. Thirty-eight animals were subjected to this experiment. The schedule of the experiment is briefly shown in table 1.

When autopsied, pathological observation of the viscera and quantitative culture of acid-fast bacilli in them were made.

The results obtained are summarized as follows:

1) For the animals (group III) receiving challenge infection but no treatment with SM, autopsy carried out one month after the infection clearly showed the protective efficacy of BCG pretreatment.

2) The animals (group I and II) receiving subcutaneous challenge infection without pretreatment with BCG showed hardly any perceptible lesions in the viscera and consequently it was difficult to judge the therapeutic effect of SM on them.

3) In the animals pretreated with BCG and treated with SM for 5 days starting on the 5th day after the subcutaneous challenge infection (groupII), the effect of SM was not as yet demonstrated at autopsy carried out one day after the last injection of SM.

4) In the animals treated with SM for 10 days starting on the 10th day after the subcutaneous challenge infection (group III), the lesion in the viscera were milder than in the untreated animals. But among the treated animals no difference in severity of lesions was found between those receiving BCG pretreatment and the others.

2. FUNDAMENTAL STUDIES IN CHEMOTHERAPY OF TUBERCULOSIS

Part 64. Studies on the Effect of SM-treatment upon Tuberculous Guinea Pigs Pretreated with BCG (No. 2)

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In the preceding paper, the auther reported the results of a series of experiments concerning the effect of SM upon guinea pigs infected with human tubercle bacilli "$H_{37}Rv$" after pretreatment with BCG.

In this paper, further experiments in this line of work were shown in table 1. The present series of experiments differs from the preceding series in the following respects:

The challenge infection was ctarried out (1) 90 days instead of 30 days after BCG inoculation, (2) by intravenous route instead of subcutaneous route and (3) with 0.25 mg instead of 0.1 mg of $H_{37}Rv$.

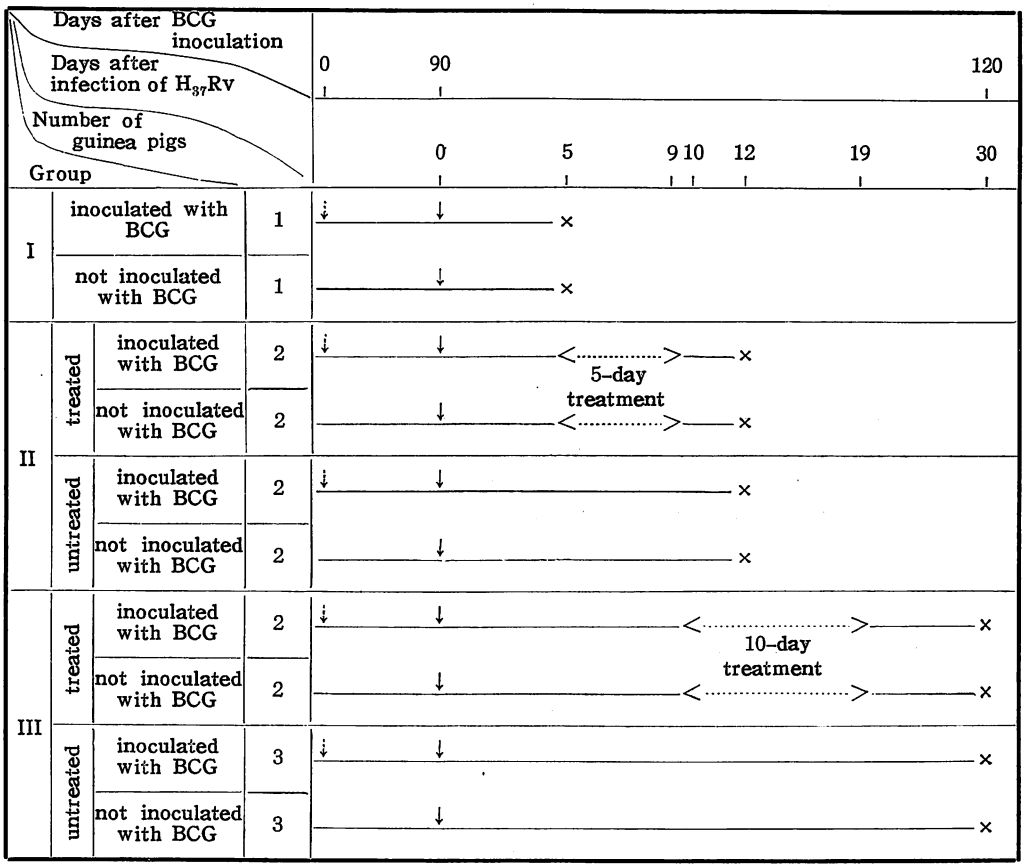
The results obtained are summarized as follows:

1) For the animals receiving no treatment with SM(group III), autopsy carried out one month after the challenge infection clearly showed the protective efficacy of BCG pretreatment just as in the preceding series. Furthermore in animals treated with SM (group III), the lesions produced in the animals pretreated with BCG were observed to be somewhat slighter than those produced in the others.

2) In the animals autopsied 12 days after the challenge infection, SM-treatment, which was given for 5 days starting on the 5th day after the challenge infection, was observed to be effective both in the cases pretreated with BCG and in the unpretreated cases. But the therapeutic effect of SM did not seem to benefit much from BCG pretreatment.

3) With the animals of groups II and III not treated with SM, the number of acid-fast bacilli in the viscera measured by quantitative culture was found to be smaller in those

Table 1. Schedule of experiment



1) ↓ inoculated with 0.1 mg of BCG
↓ infected intravenously with 0.25 mg of human tubercle bacilli "$H_{37}Rv$"
2) <·····> daily dose of SM : 40 mg
3) × autopsied

animals receiving BCG pretreatment than in the others. With those treated with SM, the animals of group II receiving BCG gave larger number of colonies in the culture than the others of the same group, while all the animals of group III gave very small numbers of colonies whether they had received BCG or not.

Conclusion from Experiments No. 1 and No. 2

1) In the guinea pigs pretreated with BCG 90 days before intravenous challenge infection with human tubercle bacilli "$H_{37}Rv$" and treated with SM for 10 days starting on the 10th day after the challenge infection and autopsied 10 days after the termination of the SM-treatment, BCG-immunization and SM-treatment were found to be cooperative in their therapeutic effect. The same result was obtained in the cases in which pretreatment with BCG was carried out 30 days before subcutaneous challenge infection.

2) On the contrary, in the guinea pigs pretreated with BCG 90 days before intravenous challenge infection and treated with for 5 days starting on the 5th day after the challenge infection and autopsied 3 days after the termination of the SM-treatment, BCG-immunization and SM-treatment did not prove cooperative.

3. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

Part 6. Studies on the Tubercle Bacilli Isolated from Sputa Collected from Street Surface
No. 2. Biological Examination

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Nine strains of acid-fast bacilli isolated from sputa on the street were submitted to biological test such as boilling-, catalase-, urease-and neutral red-tests and the results indicated that all of them were virulent.

Moreover, some of them were found to be resistant against streptomycin (100γ/ml), though they were all sensitive to other antituberculous agents.

4. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

Part 6. Studies on the Tubercle Bacilli Isolated from Sputa Collected from Street Surface
No. 3. Examination of the Virulence upon Mice

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The virulence of nine strains of acid-fast bacilli isolated from sputa on the street was tested on mice by means of intravenous, intraperitoneal, intranasal and intracranial infection.

The results obtained are summarized as follows:

1) All the strains were found to be as virulent as $H_{37}Rv$, when the infection was carried out by the intravenous route.

2) The order of virulence of all strains was found to be almost same whichever route of infection was employed.

3) Virulence of the strains was most clearly demonstrated when the infection was carried out by the intraperitoneal route.

5. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

Part 6. Studies on the Tubercle Bacilli Isolated from Sputa Collected from Street Surface

No. 4. Therapeutic Effect of Antituberculous Agents upon Mice Infected with the Bacilli

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The effect of various antituberculous agents was examined upon mice infected with a SM-resistant strain isolated from sputa on the street and $H_{37}Rv$ strain individually. The agents employed were SM, PAS, INAH, Tb 1, VM, PZA and o-Aminophenol (OM).

The results obtained are summarized as follows:

1) SM and INAH were most effective upon mice infected with $H_{37}Rv$.

2) INAH was most effective upon mice infected with the SM-resistant strain.

3) One mg of OM was equal to five mg of PAS in effectiveness when administered with some other drug.

4) SM was found to be effective to some extent upon mice infected with SM-resistant strain.

5) INAH+OM or INAH+PAS was found to be most effective upon mice infected with the SM-resistant strain and VM+OM and VM showed effectiveness equal among themselves but somewhat less than that of the first-mentioned two pairs.

6. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

Part 6. Studies on the Tubercle Bacilli Isolated from Sputa Collected from Street Surface

No. 5. Change of Drug-sensitivity of the Bacilli in the Course of Treatment of the Infected Mice with Antituberculous Drugs

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Four strains of tubercle bacilli—1 sensitive and 3 resistant to SM—isolated from sputa on the street were examined as to the change of the drug-sensitivity *in vivo*

The results obtained are summarized as follows:

1) SM-resistance of the bacilli was not observed to change by one passage through a mouse.

2) SM-resistance of the bacilli was not observed to change by the administration of daily doses of SM, PAS, INAH, Tb 1 or OM.

3) Successive passage of the bacilli through mice was carried out and it was observed (1) that the strain sensitive to SM became resistant to SM, but not to OM, PAS and INAH by administration of SM, SM+PAS, SM+OM, INAH, INAH+PAS or INAH+OM

and (2) that the strain resistant to SM (100γ/ml) became resistant to VM by administration of VM, VM+PAS, VM+OM, INAH, INAH+PAS or INAH+OM, but on the other hand the SM-resistance was weakened by administration of VM, VM+PAS or VM+OM.

7. STUDIES ON THE RESISTANCE OF MICROORGANISMS TO VARIOUS CHEMICALS

Part 7. Virulence of Drug-resistant Tubercle Bacilli upon Mice

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In order to compare the virulence of human tubercle bacilli "H_2" (susceptive parent), streptomycin- and isonicotinic acid hydrazide-resistant "H_2" strains upon mice, subcutaneous, intravenous, intraabdominal and intranasal infections were carried out. A number of mice were autopsied at the end of each week, from the first to the fourth, after the infection, and pathological examination of the viscera and quantitative culture of the bacilli in them were performed.

The results obtained are summarized as follows:

1) By intranasal infection, lesions were found to develope only in the lung and also the bacilli could be cultivated only from the lung. All the three strains produced almost equal results as to the grade of the lesions and number of the bacilli measured by quantitative culture.

2) The animals infected intravenously with INAH-resistant strain showed most serious pulmonary lesions and gave largest bacillus counts in the viscera four weeks after the infection.

3) Subcutaneous infection of any strain, produced practically no lesions in the viscera and quantitative culture of the bacilli also gave negative results.

8. IMMUNOLOGICAL STUDIES ON ERYTHROCYTES SENSITIZED WITH OLD TUBERCULIN

Part 3. On the Immunological Antigenicity of Erythrocytes Sensitized with Old Tuberculin (III)

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In the preceding paper, it was pointed out by the author that OT-sensitized red cells of rabbit were active in producing hemagglutinin and hemolysin against OT-sensitized red cells and the complement fixation antibody against OT.

In this paper, the data of the further experiments in this line of work are reported concerning precipitation reaction on a serum obtained from rabbits injected with OT-sensitized red cells. As antigens in reaction, OT, polysaccharide and protein fractions from OT and tubercle bacilli were employed.

The results obtained are summarized as follows:

1) The antiserum and the various antigens gave positive results in the pretipitin test.

2) The polysaccharide fraction of OT was clearly demonstrated to be different from that of tubercle bacilli in antigenic activity.

3) No clear-cut difference of antigenicity was demonstrated between the protein fraction from OT and that from tubercle bacilli.

In passing, antiserum obtained from a rabbit receiving OT singly was seen to give positive results in precipitation tests with the various antigens described above.

9. IMMUNOLOGICAL STUDIES IN TUBERCULOSIS

Part 7. Use of Twins in Analytical Studies of Nature-nurture Correlations Concerning Resistance to Tuberculosis

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For the purpose of investigating the influence of heredity and environment upon the development of tuberculin allergy and immunity against tuberculosis, 29 pairs of identical and 24 pairs of fraternal twins were subjected to this experiment.

There are some physical traits about which identical twins show much more marked similarity than fraternal twins. In such traits heredity must be the dominant factor.

From this point of view, the results obtained are summarized as follows:

Heredity is the dominant factor in determining (1) the strength of tuberculin allergy developed by BCG inoculation and natural infection, (2) the strength of the phagocytic power of leucocytes and the growth inhibitory power of whole blood against BCG and especially human tubercle bacilli "H_{37} Rv", (3) the leucocyte count and neutrophilic leucocyte count, (4) the erythrocyte sedimentation rate, (5) height, standing and sitting, and (6) transversal diameter of the heart in chest-roentogenogram.

10. FORMATION OF TUBERCULIN BY WASHED TUBERCLE BACILLI IN CITRATE SOLUTION

Part 10. Study on the Influence of Medium upon the Citrate-Tuberculin Producing Property of Tubercle Bacilli

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The present paper describes the results of comparative experiments concerning the influence of media on the "citrate-tuberculin"-producing property of washed tubercle bacilli.

The media used were, glycerol-broth (pH6.8), and three synthetic media, Long's, Sauton's, and Dorset's.

Four sets of flasks, each flask in each series containing 50 ml of one of these media,were simultaneously inoculated with human tubercle bacillus, Aoyama B, and incubated at 37° C for a period of time. At the end of incubation, the bacilli harvested from two cultures in each series were washed thorougly, immersed in 20 ml of 0.1 M citrate solution, and incubated at 37°C for 24 hours, at the end of which time the citrate solutions separated from the organisms were passed through Seitz filters.

Thus, four sets of samples of citate-tuberculin (CIT), each of which was obtained from the organisms grown on glycerol-broth, or Long's, Sauton's, or Dorset's medium, were tested for their tuberculin potency upon tuberculous guinea pigs, 0.1 ml of various dilutions of the respective samples being simultaneously injected intradermally into the same animal.

The comparative skin tests carried out with these four sets of samples of CIT revealed that all the samples obtained from the bacilli grown on Dorset's medium were as highly potent in eliciting tuberculin skin reaction as those obtained from the bacilli grown on Sauton's medium, while the samples obtained from the bacilli grown on glycerol-broth or Long's medium were found to be only about one-tenth or one-twentieth as active as the former.